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(54) Transesterification of fats and oils

(57) Alkyl esters are prepared from fats and oils and glyceride-containing fractions thereof by mixing a solution of the fat or oil in an inert organic solvent with an alkanol in the presence of a lipase and water. Usually, the oil, alkanol and solvent are mixed together and the mixture added to a paste or suspension of the lipase in the water. The process has particular application to vegetable oils and especially to palm oil. Preferably the amount of water and alkanol is at least 2 litres per kilogram of oil and the inert solvent, e.g. petroleum ether, is used in similar amounts.

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SPECIFICATION

Transesterification of fats and oils

The present invention relates to the transesterification (ie. alcoholysis) of fats and oils and of glyceride-containing fractions thereof. It has particular, but not exclusive, application to palm oil and palm oil fractions. Except where it is clear from the context that a true oil is intended, the work 'oil' is used hereinafter to include both fats and oils.

Oils contain primarily fatty acid glycerides and, usually, free fatty acids. The free fatty acid content depends upon the source of the oil and, in the case of fractions thereof, the treatment by which the fraction has been obtained. In general terms, the free fatty acid content interferes with the refining of the oil and/or reaction of the glyceride content of the oil and therefore the free fatty acid content usually is removed at an early stage in the refining of the oil.

It is known to prepare alkyl esters by esterification of the free fatty acid content of oils and/or transesterification of the glyceride content of the oils. The production of alkyl esters from oils, especially vegetable oils, is becoming of increasing commercial and political importance. Generally, the alkyl esters are useful chemical feedstock for the production of, for example, biodegradable detergents. The methyl esters are potential diesel substitutes and hence of particular interest to countries having an ample supply of vegetable oil but little or no fossil fuel deposits. Accordingly, there is substantial interest in developing an efficient process for the preparation of alkyl esters from vegetable oils.

As far as we are aware, all previously proposed processes for the preparation of alkyl esters from oil involve two steps. In the first step, the free fatty acid content of the oil is removed or the glyceride content is hydrolysed to free fatty acid and glycerol. Removal of the free fatty acid usually is by alkali refining or esterification to alkyl esters. The hydrolysis usually is by treatment of the oil with steam at high temperature (about 260°C) although, as explained below, ambient temperature enzyme hydrolysis has been proposed. When the free fatty acid is removed in the first step, the second step is transesterification of the glyceride content, usually by alkali catalysed transesterification. However, when the first step is hydrolysis, the second step is esterification of the free fatty acid content of the hydrolysed product.

It is known that lipase enzymes catalyse both hydrolytic and esterification reactions and have particular application to the treatment of oils. Lipolitic enzymes have been used for nearly 100 years for fat-splitting and the use of lipase in the hydrolysis, interesterification (ie. acidolysis) and transesterification (ie. alcoholysis) of oils has been extensively investigated in recent years (see, for example, U.K. Patent Specification Nos. 2042579, 2035359, 1577933 and 1403847; European Patent Specification No. 0126416; Japanese Patent Specification Nos. 59091889, 57111398, 57057799 and 51061673; and International Patent Specification No. WO 82/03873). The use of a lipase as a catalyst in hydrolytic or esterification reactions offers the substantial advantage of being able to carry the reaction at ambient pressures and at, or a little above, ambient temperatures. However, in using a lipase with oils, care has had to be taken to control the water content of the reaction medium. In order to be fully activated, the lipase should be fully hydrated but the presence of water favours hyrolysis rather than esterification. This militates against the use of lipase in the transesterification of oils and we are not aware of any proposal to transesterify an oil with an alkanol in the presence of an excess by weight of water over the substrate (ie. oil).

U.K. Patent Specification No. 2042579 discloses the production of glycerides by interesterification of a glyceride mixture in the presence of a lipase and of a di- or tri- hydric alcohol. The glyceride mixture comprises either (a) at least two glycerides or (b) at least one glyceride or at least one fatty acid. The amount of alcohol employed is small, preferably 0.1 to 10% by weight of the raw glyceride mixture and there is no reference to the use of any monohydric alcohol. The interesterification preferably is carried out in the absence or substantial absence of water. Specific reference is made to the presence of palm oil as a component of the glyceride mixture.

U.K. Patent Specification No. 2035359 discloses a method of producing cocoa butter substitute by increasing the proportion of stearic and/or palmitic acid residues at the 1 and 3 positions of the triglycerides of an oil by interesterification in the presence of a lipase having specificity for the 1 and 3 positions of triglycerides. The starting material is an oil containing a large proportion of triglycerides having an oleic acid residue at the 2 position and specific reference is made to the use of palm oil. The reaction is carried out under substantially anhydrous conditions, ie. not more than 0.18% by weight of water.

U.K. Patent Specification No. 1577933 discloses the re-arrangement of fatty acid groups in oils by contacting the oil with a lipase and a small amount, i.e. less than about 10%, of water. Preferably the water content is 0.2 to 1%.

U.K. Patent Specification No. 1403847 discloses that a particular lipase (designated 26466 RP) successively liberates the three fatty acid residues of a triglyceride by hydrolysis.

European Patent Specification No. 0126416 discloses a two step lipase treatment of oils. The

first step is dominated by hydrolysis of triglyceride to diglyceride. The second step is dominated by esterification of the diglyceride hydrolysis product with added fatty acid to produce triglyceride. Alcohol, preferably C_4–C_{18} alkanol, can be added during either step to esterify the free fatty acid hydrolysis product in order to facilitate recovery of triglyceride. The optimum water content during the hydrolysis step is 2 to 10% by weight of the oil and it is preferred to reduce the water content before esterification. Specific reference is made to the treatment of palm butter and palm olein.

Japanese Patent Specification No. 59091889 discloses the decomposition of oils with a lipase in the presence of water-insoluble organic solvents. The preferred water content is 50 to 300 percent by weight of the oil. The specified oils include palm oil.

Japanese Patent Specification No. 57111398 discloses the use of lipase to cause ester-interchange in a solubilised oil. Preferably, the oil is solubilised in an aqueous solution containing a water-soluble polyhydroxy compound, especially a sugar or sugar alcohol. The concentration of polyhydroxy compound preferably is at least 35 percent by weight.

Japanese Patent Specification No. 57057799 discloses the hydrolysis of an oil by mixing it in its molten state with a cooled aqueous solution of a lipase to obtain a stable dispersion at a temperature below the melting point of the oil, in which state it is hydrolysed to fatty acid and glycerol. The aqueous lipase solution constitutes 10 to 30 percent by weight of the oil and specific reference is made to the treatment of palm oil.

Japanese Patent Specification No. 51061673 discloses the preparation of aromatic fatty esters for use as flavours by decomposing an oil with a lipase and then adding a monohydric alcohol to the reaction product to esterify the resultant fatty acid content. Water may be present in an amount of 10 to 90 percent but preferably is about 50 percent. Specific reference is made to the treatment of palm oil and palm kernel oil.

International Patent Specification No. WO 82/03783 discloses the preparation of organic compounds which are susceptible to hydrolysis by contacting a dispersion of reactants in a water-immiscible organic liquid with an enzyme activated with water and with desiccant means to lower the water activity of dispersion. It is stated that a particular application of the process is in the enzymic interesterification, with or without added free fatty acid, of oils. Specific reference is made to the use of a lipase and to the treatment of palm oil. Reference is made to the addition of glycerol to combine with free fatty acid present to form partial glycerides.

Having regard to the prior art acknowledged above, the Inventors have now surprisingly found alkyl esters can be obtained in good yield from oils by mixing a solution of the oil in an inert organic solvent with an alkanol in the presence of a lipase and an excess (by weight) of water over the substrate (ie. oil or oil fraction). This procedure causes alcoholysis of the glyceride content of the oil and also esterification of the free fatty acid content. It is particularly surprising that the procedure is effective when using methanol or ethanol as the alkanol.

According to the present invention, there is provided a process for the preparation of an alkyl ester from a substrate selected from oils (as hereinbefore defined) and glyceride-containing fractions thereof, which comprises mixing a solution of the substrate in an inert organic solvent with an alkanol in the presence of a lipase and an excess (by weight) of water over the weight of substrate.

The substrate can be an animal, synthetic or, preferably, vegetable oil or a glyceride-containing fraction thereof. Examples of animal oil are lard, fish oil and, especially, tallow and examples of synthetic oils are tristearin and triolein. Examples of vegetable fats and oils are coconut oil, corn oil, rapeseed oil, soyabean oil, sunflower oil, and especially, palm oil. Examples of glyceride-containing fractions are fatty acid distillates obtained by distillation of vegetable oils, sludge oils derived from the refining and milling of vegetable oils, and refined oils.

As mentioned previously, the invention has particular application to vegetable oils and especially to palm oil. In particular, the process provides alkyl esters in good yield from crude palm oil, crude palm kernel oil, crude palm stearin, palm fatty acid distillate, palm sludge oil, and refined, bleached and deodorized (RBD) palm oil. These oils differ substantially in their free fatty acid (FFA) content. Typically, their FFA content is as follows:–

Oil	FFA
RBD Palm Oil	less than 0.1%
Crude Palm Oil	1-10%
Palm Fatty Acid Distillate	70-90%
Crude Palm Kernel Oil	1-10%
Crude Palm Stearin	1-10%
Palm Sludge Oil	10-80%

Glycerides are immiscible with water and slightly miscible with lower alkanols and therefore it is necessary to conduct the esterification in the present of an organic solvent for the glyceride-containing substrate. The solvent should be substantially immiscible with water and suitable solvents include hyrocarbons, ethers and esters. Presently preferred solvents are methyl esters of palm oil and, especially, petroleum ether. The presently most preferred solvent is 60–80°C petroleum ether.

Sufficient solvent must be present to at least partially solubilise the substrate in the reaction medium. Usually, the solvent will be present in an excess by weight of the substrate. Suitably, the solvent will be present in an amount of at least 2 litres per kilogram of substrate. An amount of 2 to 10 litres, especially 4 to 6 litres, solvent per kilogram substrate has been found to be satisfactory.

The water is present in an excess by weight of the substrate. Usually, at least 2 litres of water and alkanol (combined volume) will be present per kilogram of substrate. Suitable, 15 to 40 litres, especially 20 to 30 litres, of water and alkanol (combined volume) per kilogram substrate is employed.

The water content should exceed 40% by volume and preferably exceed 50% by volume of the combined water and alkanol content. Suitably, the water content is in the range 50% to 90% by volume.

The alkanol preferably has a carbon content of 1 to 8 carbon atoms arranged in a straight or branched chain configuration. Examples of such alkanols are methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, and n-octanol. The preferred alkanol is methanol because methyl esters of fatty acids are of particular use as diesel substitutes.

The process of the invention usually will be carried out at temperature within the range 20 to 50°C. In the tropical and subtropical countries in which palm oil is harvested, the ambient temperature is likely to be between 25 and 35°C and hence, when the substrate is palm oil or a fraction thereof, the reaction will usually be carried out without heating. However, in the United Kingdom and Northern Europe, it will usually be desirable to warm the reaction mixture to a temperature within the range 25 to 40°C. Lipases usually exhibit their maximum activity at a temperature in the range 35 to 40°C and hence temperatures above 40°C are unlikely to be required for most purposes. Higher temperatures can be used if required, for example in order to improve the fluidity of the reaction mixture, especially when the substrate is a fat.

Lipases are a group of esterases and it is known that members of the group differ in their activity and selectively. However, it is a matter straight forward trial and error experimentation in order to determine the suitability of any particular lipase for use in the process of the present invention. At the present time, it is preferred that the lipase is of the Candida group, especially Candida Cylindracea. As will be noted from the experimental data provided later in this specification, Candida Cylindracea has been found to perform significantly better than Lipase 3A (a commercially available lipase from Novo Industri A/S).

In large scale industrial application of the process of this invention, it is preferred that the lipase is fixed, ie. immobilised, by adsorption on a carrier. A wide range of carriers are known to be suitable for fixing lipase. These include Celite (Trade Mark), active carbon, ion exchange resins, Florisil (Trade Mark), silica-gel and alumina. Acid-treated Florisil (magnesium silicate) has been found to be a particularly suitable lipase carrier for use in the process of the present invention. The acid treatment can be performed by the method of K.K. Carroll (JAOCS, 40 (1963), 413–419). In this method, Florisil is boiled in concentrated hydrochloric acid at 100°C for prolonged periods and then water-washed until neutrality. The neutral Florisil is then sequenti-

ally washed with methanol, chloroform and ether and subsequently activated by heating overnight at 100°C. However, we have found that, for the purposes of the present invention, it is sufficient to suspend the Florisil in concentrated hydrochloric acid overnight at, for example, 28–30°C (Malaysian room temperature) and then water-wash until neutrality. The neutral Florisil is then dried with a suction pump and activated by heating for two hours at 180°C.

Usually, the oil, alkanol and solvent will be mixed together and the mixture added to the paste or suspension of the lipase in the water.

The invention is illustrated in the following non-limiting examples.

Example 1

Preparation of methyl esters by methanolysis of palm oil and palm oil fractions

Refined, bleached and deodorized (RDB) palm oil (0.1g) was weighed into a small vial (about 1.5cm diameter and 5cm height) and methanol (1ml) was then added, followed by 60–80 petroleum ether (0.2ml). Water (1ml) and lipase (Candida Cylindrecea; 0.05g) was added to a second vial (also of about 1.5cm diameter and 5cm height) to form a paste. The oil/methanol/petroleum ether mixture was added to the lipase/water paste in the second vial and the mixture shaken for about 20 hours at Malaysian temperature (28–30°C). The reaction product was then extracted successively twice with petroleum ether and twice with chloroform and the extract analysed for its methyl ester content.

The procedure reported above was repeated with crude palm oil containing 20% free fatty acid (FFA); palm fatty acid distillate containing 80% FFA; palm kernel oil, crude palm stearin containing 3% FFA; and palm sludge oil containing 76% FFA as substrates. In each case, the amount of petroleum ether employed was 0.5ml instead of 0.2ml as used with RBD palm oil as the substrate. Further, in the case of palm kernel oil and crude palm stearin, an increased amount of water and decreased amount of methanol was used. With palm kernel oil as the substrate, the amount of water was 1.25ml and the amount of methanol was 0.75ml. With crude palm stearin as the substrate, 2.7ml water and 0.3ml ethanol was employed.

In some procedures, the mixture was shaken using a magnetic stirrer whilst in others an electrical shaker was used.

The yields of ester obtained were as follows:–

Oil	Ester Yield
RBD Palm Oil	98%
20% FFA Palm Oil	95%
80% FFA Palm Fatty Acid Distillate	97%
6% FFA Palm Kernel Oil	99%
3% FFA Crude Palm Stearin	99%
76% FFA Palm Sludge Oil	80%

Table 1 below sets out in tabulated form experimental data and yields obtained.

TABLE 1

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT*	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	0.05	P.E.	0.2
PALM OIL	0.1	20%	0.05	P.E.	0.5
PALM DISTILLATE	0.1	80%	0.05	P.E.	0.5
PALM KERNEL	0.1	6%	0.05	P.E.	0.5
PALM STEARIN	0.1	3%	0.05	P.E.	0.5
PALM SLUDGE	0.1	76%	0.05	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	1	Methyl	1	1 : 1	20	98%
PALM OIL	1	Methyl	1	1 : 1	20	95%
PALM DISTILLATE	1	Methyl	1	1 : 1	20	97%
PALM KERNEL	1.25	Methyl	0.75	1.6 : 1	20	99%
PALM STEARIN	2.7	Methyl	0.3	9 : 1	20	99%
PALM SLUDGE	1	Methyl	1	1 : 1	20	80%

* P.E=60–80°C Petroleum Ether

Example 2

Preparation of methyl esters by methanolysis of animal fat and vegetable oils

The procedure of Example 1 was repeated using tallow, rapeseed oil, sunflower oil, coconut oil, crude corn oil and soyabean oil as substrates instead of palm oil and its fractions. In each case, 0.5ml petroleum ether was used. Except when using coconut oil and soyabean as substrate, the amount of water used was 2.7ml and the amount of methanol used was 0.3ml. When using coconut oil and soyabean oil as substrate, 1.25ml water and 0.75ml methanol was employed. The yields of methyl ester obtained were as follows:–

Oil	Ester Yield
Tallow (3.9% FFA)	99%
Rapeseed Oil (3.2% FFA)	98%
Sunflower Oil (3.5% FFA)	98%
Coconut Oil (1.5% FFA)	99%
Crude Corn Oil (9.6%% FFA)	98%
Soyabean Oil (1.3% FFA)	70%

Table 2 below sets out in tabulated form experimental data and yields obtained.

TABLE 2

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
TALLOW	0.1	3.9%	0.05	P.E.	0.5
RAPESEED	0.1	3.2%	0.05	P.E.	0.5
SUNFLOWER	0.1	3.5%	0.05	P.E.	0.5
COCONUT	0.1	1.5%	0.05	P.E.	0.5
CORN	0.1	9.6%	0.05	P.E.	0.5
SOYABEAN	0.1	1.3%	0.05	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
TALLOW	2.7	Methyl	0.3	9 : 1	20	99%
RAPESEED	2.7	Methyl	0.3	9 : 1	20	98%
SUNFLOWER	2.7	Methyl	0.3	9 : 1	20	98%
COCONUT	1.25	Methyl	0.75	1.6: 1	20	99%
CORN	2.7	Methyl	0.3	9 : 1	20	98%
SOYABEAN	1.25	Methyl	0.75	1.6: 1	20	70%

Example 3

Preparation of Ethyl Esters by ethanolysis of Palm Oil and RBD Palm Oil

The procedure of Example 1 was repeated using RBD palm oil and 20% FFA palm oil as substrates and ethanol instead of methanol. In each case, 0.5ml petroleum ether, 0.3ml ethanol and 2.7ml water were used. The yields of ethyl esters were as follows:–

Oil	Ester Yield
RBD Palm Oil	100%
20% FFA Palm Oil	99%

Table 3 below sets out in tabulated form experimental data and yield obtained.

TABLE 3

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	0.1	P.E.	0.5
PALM OIL	0.1	20%	0.1	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	2.7	Ethyl	0.3	9 : 1	20	100%
PALM OIL	2.7	Ethyl	0.3	9 : 1	20	99%

Example 4

Effect of Water : Ethanol Ratio on preparation of Ethyl Esters by Ethanolysis of RBD Palm Oil

The procedure of Example 1 was repeated using RBD palm oil as the substrate but varying the proportions of ethanol and water. In each case, the sum of the ethanol and water content was 3ml but the water : ethanol ratio varied from 0:1 to 9:1 as specified below. The yields of ethyl ester obtained were as follows:

Water : Ethanol Ratio	Ester Yield
0:1	0%
0.05:1	2%
0.1:1	3%
0.33:1	10%
1:1	30%
3:1	70%
9:1	95%

Table 4 below sets out in tabulated form experimental data and yields obtained.

TABLE 4

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	0.05	P.E.	0.5
PALM RBD	0.1	None	0.05	P.E.	0.5
PALM RBD	0.1	None	0.05	P.E.	0.5
PALM RBD	0.1	None	0.05	P.E.	0.5
PALM RBD	0.1	None	0.05	P.E.	0.5
PALM RBD	0.1	None	0.05	P.E.	0.5
PALM RBD	0.1	None	0.05	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	2.7	Ethyl	0.3	9 : 1	20	95%
PALM RBD	2.25	Ethyl	0.75	3 : 1	20	70%
PALM RBD	1.5	Ethyl	1.5	1 : 1	20	30%
PALM RBD	0.75	Ethyl	2.25	0.33 : 1	20	10%
PALM RBD	0.3	Ethyl	2.7	0.1 : 1	20	3%
PALM RBD	0.15	Ethyl	2.85	0.05 : 1	20	2%
PALM RBD	None	Ethyl	3	0 : 1	20	0%

Example 5
Preparation of Isopropyl Esters by Alkanolysis of Palm Oil and Palm Oil Fractions with Isopropyl Alcohol

The procedure of Example 1 was repeated using RBD palm oil, 20% FFA palm oil and 80% FFA palm fatty acid distillate as substrates and isopropyl alcohol instead of methanol. In each case, 0.5ml petroleum ether was used as the solvent. When the substrate was RBD palm oil or 20% FFA palm oil, the amount of water used was 2.7ml and the amount of isopropyl alcohol was 0.3ml. When the substrate was palm fatty acid distillate, 1ml of water and 1ml of isopropyl alcohol were employed. Further, the amount of lipase was increased to 0.1g when using 20% FFA palm oil as the substrate. The ester yields were as follows:–

Oil	Ester Yield
RBD Palm Oil	99%
20% FFA Palm Oil	99%
80% FFA Palm Fatty Acid Distillate	90%

Table 5 below sets out in tabulated form experimental data and yields obtained.

TABLE 5

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	0.05	P.E.	0.5
PALM OIL	0.1	20%	0.1	P.E.	0.5
PALM DISTILLATE	0.1	80%	0.05	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	2.7	i-propyl	0.3	9 : i	20	99%
PALM OIL	2.7	i-propyl	0.3	9 : 1	20	99%
PALM DISTILLATE	1.0	i-propyl	1.0	1 : 1	20	90%

Example 6

Preparation of n-butyl Esters by Alkanolysis of RBD Palm Oil using n-butanol

The procedure of Example 1 was repeated using RBD palm oil as a substrate with 0.5ml petroleum ether, 1ml butanol and 2ml water for 19 hours reaction time. The yield of butyl esters was 100%.

Table 6 below sets out in tabulated form experimental data and yield obtained.

TABLE 6

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	0.05	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	2.0	n-butyl	1.0	2 : 1	19	100%

Example 7

Effect of Solvent on preparation of Methyl Esters by Methanolysis of RBD Palm Oil

The procedure of Example 1 was repeated using RBD palm oil as the substrate with no solvent, 0.2ml petroleum ether or 0.5ml methyl esters of crude palm oil. In each case, 1ml of methanol was used and, except when there was no solvent, 1ml of water also was used. When there was no solvent, only 0.5ml of water was employed. There was slight variation in the reaction time (see Table 7 below). The yields of methyl esters were as follows:–

Solvent	Ester Yield
Methyl Esters	95%
Petroleum Ether	98%
None	20%

Table 7 below sets out in tabulated form experimental data and yields obtained.

TABLE 7

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT*	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	0.05	MeE	0.5
PALM RBD	0.1	None	0.05	P.E.	0.2
PALM RBD	0.1	None	0.05	None	–

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	1.0	Methyl	1.0	1 : 1	22.5	99%
PALM RBD	1.0	Methyl	1.0	1 : 1	18.25	98%
PALM RBD	0.5	Methyl	1.0	0.5 : 1	25.25	20%

* MeE=Methyl esters of crude palm oil

Example 8
Preparation of Methyl Esters by Methanolysis of Palm Oil and Palm Oil Fractions with immobilised Lipase

The procedure of Example 1 was repeated using RBD palm oil, 30% FFA palm oil, and crude kernel oil as substrates. In each case, 0.5ml petroleum ether was used as the solvent. The lipase was immobilised on Florisil and used in an amount of 1g (RBD palm oil) or 0.5g (30% FFA palm oil and crude palm kernel oil). The amounts of methanol and water used were 1.0ml and 0.3ml respectively (RBD palm oil), 2.7ml and 0.3ml respectively (30% FFA palm oil), and 1.3ml and 0.4ml respectively (crude palm kernel oil). The yields of methyl esters were as follows:–

Oil	Ester Yield
RBD Palm Oil	99%
30% FFA Palm Oil	70%
6% FFA Crude Palm Kernel Oil	70%

Table 8 below sets out in tabulated form experimental data and yields obtained.

The immobilized lipase was prepared in the manner described below from acid-treated Florisil and Candida Cylindracea.

Acid-treated Florisil (0.5g) was added to a solution of the lipase (100mg) in 0.1m TRIS–HCl buffer (5ml, pH 7.5) in a conical flask. The flask was shaken at Malaysian room temperature for 10 minutes at 200 rpm on a rotary shaker. The mixture was then filtered through a sintered

glass filter and the residue washed repeatedly with water until no appreciable amount of protein was removed by further washing. The immobilised lipase so prepared was used immediately or stored at 4°C for up to 5 days with little loss of activity.

The acid-treated Florisil was obtained by suspending Florisil in concentrated hydrochloric acid overnight at room temperature with gentle stirring. It was then thoroughly washed with water until neutral. The wet acid-treated Florisil was dried with a suction pump and then heated for two hours at 180°C.

TABLE 8

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE* (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	1 (Im)	P.E.	0.5
PALM OIL	0.1	30%	0.5 (Im)	P.E.	0.5
PALM KERNEL	0.1	6%	0.5 (Im)	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	1.0	Methyl	0.3	3.3 : 1	20	99%
PALM OIL	2.7	Methyl	0.3	9 : 1	20	70%
PALM KERNEL	1.3	Methyl	0.4	3.2 : 1	20	70%

* Im=immobilized on acid-treated Florisil

Example 9

Preparation of Alkyl Esters by Alkanolysis of RBD Palm Oil using Immobolised lipase

The procedure of Example 8 was repeated using RBD palm oil as the substrate and methanol, ethanol or isopropyl alcohol as the alkanol. In each case, 1g immobilise lipase was used with 0.5ml petroleum ether, 0.3ml alkanol and 1ml water. The yields of esters were as follows:–

Alcohol	Ester Yield
Methanol	99%
Ethanol	90%
Isopropyl Alcohol	97%

Table 9 below sets out in tabulated form experimental data and yields obtained.

TABLE 9

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	1 (Im)	P.E.	0.5
PALM RBD	0.1	None	1 (Im)	P.E.	0.5
PALM RBD	0.1	None	1 (Im)	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	1	Methyl	0.3	3.3 : 1	20	99%
P RBD	1	Ethyl	0.3	3.3 : 1	20	90%
P RBD	1	i-Propyl	0.3	3.3 : 1	20	97%

Example 10

Preparation of Methyl Esters by Methanolysis of Vegetable Oils using Immobilized Lipase

The procedure of Example 8 was repeated using corn oil, rapeseed oil, soyabean and sunflower seed oil as substrates. When the substrate was soyabean oil, 0.1g of immobilised lipase was used but otherwise 0.5g immobilised lipase was employed. Except when using corn oil as the substrate, 1.3ml water and 0.4ml methanol were used. With corn oil, the amount of water was 2.7ml and the amount of methanol was 0.3ml. The yields of esters were as follows:–

Oil	Ester Yield
Corn Oil (9.6% FFA)	60%
Rapeseed Oil (3.2% FFA)	30%
Soyabean Oil (1.3% FFA)	50%
Sunflower Seed Oil (3.5% FFA)	50%

Table 10 below sets out in tabulated form experimental data and yields obtained.

TABLE 10

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
CORN	0.1	9.6%	0.5 (Im)	P.E.	0.5
RAPESEED	0.1	3.2%	0.5 (Im)	P.E.	0.5
SOYABEAN	0.1	1.3%	0.1 (Im)	P.E.	0.5
SUNFLOWER	0.1	3.5%	0.5 (Im)	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
CORN	2.7	Methyl	0.3	9 : 1	20	60%
RAPESEED	1.3	Methyl	0.4	3.2 : 1	20	30%
SOYABEAN	1.3	Methyl	0.4	3.2 : 1	20	50%
SUNFLOWER	1.3	Methyl	0.4	3.2 : 1	20	50%

Example 11

Preparation of Methyl Esters by Methanolysis of Palm Oil and Palm Oil Fractions using Lipase 3A

The procedure of Example 1 was repeated using RBD palm oil, 30% FFA palm oil, palm fatty acid distillate and crude kernel oil as substrates but with Lipase 3A (supplied by Novo Industri A/S) as the lipase. In the case of crude palm kernel oil, 0.1g lipase was used but otherwise 0.05g lipase was employed. In each case, 2.7ml water, 0.3ml methanol and 0.5ml petroleum ether were used. The yields of esters were as follows:–

Oil	Ester Yield
RBD Palm Oil	5%
30% FFA Palm Oil	80%
80% FFA Palm Fatty Acid Distillate	90%
6% FFA Crude Palm Kernel Oil	10%

TABLE 11

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE* (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	0.5 (3A)	P.E.	0.5
PALM OIL	0.1	30%	0.05 (3A)	P.E.	0.5
PALM DISTILLATE	0.1	80%	0.05 (3A)	P.E.	0.5
PALM KERNEL	0.1	6%	0.1 (3A)	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	2.7	Methyl	0.3	9 : 1	20	5%
PALM OIL	2.7	Methyl	0.3	9 : 1	20	80%
PALM DISTILLATE	2.7	Methyl	0.3	9 : 1	20	90%
PALM KERNEL	2.7	Methyl	0.3	9 : 1	20	10%

* 3A=Lipase 3A

Example 12

Preparation of Methyl Esters by Methanolysis of Vegetable Oils using Lipase 3A

The procedure of Example 11 was repeated using soyabean oil, crude corn oil and rapeseed oil as substrates. In each case, 0.05g lipase 3A was employed. The yields of esters were as follows:—

Oil	Ester Yield
Soyabean Oil (1.3% FFA)	70%
Crude Corn Oil (9.6% FFA)	60%
Rapeseed Oil (3.2% FFA)	40%

Table 12 below sets out in tabulated form experimental data and yields obtained.

TABLE 12

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
SOYABEAN	0.1	1.3%	0.05 (3A)	P.E.	0.5
CORN	0.1	9.6%	0.05 (3A)	P.E.	0.5
RAPESEED	0.1	3.2%	0.05 (3A)	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
SOYABEAN	2.7	Methyl	0.3	9 : 1	20	70%
CORN	2.7	Methyl	0.3	9 : 1	20	60%
RAPESEED	2.7	Methyl	0.3	9 : 1	20	40%

Example 13

Preparation of Alkyl Esters by Alkanolysis of RBD Palm Oil using Lipase 3A

The procedure of Example 11 repeated using RBD palm oil as the substrate and methanol, ethanol, isopropyl alcohol, n-butanol, or n-octanol as the alkanol. In each case, 0.05g Lipase 3A was employed. The ester yields obtained were only 5%.

Table 13 below sets out in tabulated form experimental data and yields obtained. As can be seen the reaction parameters were not optimal for Lipase 3A which appears to be more selective than Candida Cylindracea.

TABLE 13

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	0.1	None	0.05 (3A)	P.E.	0.5
PALM RBD	0.1	None	0.05 (3A)	P.E.	0.5
PALM RBD	0.1	None	0.05 (3A)	P.E.	0.5
PALM RBD	0.1	None	0.05 (3A)	P.E.	0.5
PALM RBD	0.1	None	0.05 (3A)	P.E.	0.5
PALM RBD	0.1	None	0.05 (3A)	P.E.	0.5

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	2.7	Methyl	0.3	9 : 1	20	5%
PALM RBD	2.7	Ethyl	0.3	9 : 1	20	5%
PALM RBD	2.7	i-propyl	0.3	9 : 1	20	5%
PALM RBD	2.7	n-butyl	0.3	9 : 1	20	5%
PALM RBD	2.7	i-butyl	0.3	9 : 1	20	5%
PALM RBD	2.7	n-octyl	0.3	9 : 1	20	5%

Example 14

Effect of Time on preparation of Methyl Esters by Methanolysis of RBD Palm Oil

The procedure of Example 1 was repeated on a larger scale using RBD palm oil (5g) as the substrate. The lipase (7.5g) and water (50ml) was added to a 500ml volumetric flask and the mixture of RBD palm oil (5g), methanol (50ml) and petroleum ether (25ml) was added. Samples were withdrawn from the flask at hourly intervals for the first six hours and then after 25 hours. In each case, the methyl ester content of the sample was determined. The ester yields were as follows:–

Reaction Time	Ester Yield
1 hour	40%
2 hours	50%
3 hours	50%
4 hours	70%
5 hours	80%
6 hours	90%
25 hours	99%

Table 14 below sets out in tabulated form experimental data and yields obtained.

TABLE 14

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	5	None	7.5	P.E.	25
PALM RBD	5	None	7.5	P.E.	25
PALM RBD	5	None	7.5	P.E.	25
PALM RBD	5	None	7.5	P.E.	25
PALM RBD	5	None	7.5	P.E.	25
PALM RBD	5	None	7.5	P.E.	25
PALM RBD	5	None	7.5	P.E.	25

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	50	Methyl	50	1 : 1	1	40%
PALM RBD	50	Methyl	50	1 : 1	2	50%
PALM RBD	50	Methyl	50	1 : 1	3	50%
PALM RBD	50	Methyl	50	1 : 1	4	70%
PALM RBD	50	Methyl	50	1 : 1	5	80%
PALM RBD	50	Methyl	50	1 : 1	6	90%
PALM RBD	50	Methyl	50	1 : 1	25	99%

Example 15

Effect of Time on preparation of Methyl Esters by Alkanolysis of RBD Palm Oil with n-butyl

The procedure of Example 14 was repeated using n-butyl (50ml) instead of methanol and with a reduced amount of lipase (2.5g) and an increased amount of water (100ml). The yields of ester were as follows:–

Reaction Time	Ester Yield
1 hour	20%
2 hours	30%
3 hours	50%
4 hours	60%
5 hours	60%
6 hours	70%
7 hours	80%
25 hours	100%

Table 15 below sets out in tabulated form experimental data and yields obtained.

TABLE 15

OIL	AMOUNT OF OIL (g)	FFA (%)	LIPASE (g)	SOLVENT	SOLVENT AMOUNT (ml)
PALM RBD	5	None	2.5	P.E.	25
PALM RBD	5	None	2.5	P.E.	25
PALM RBD	5	None	2.5	P.E.	25
PALM RBD	5	None	2.5	P.E.	25
PALM RBD	5	None	2.5	P.E.	25
PALM RBD	5	None	2.5	P.E.	25
PALM RBD	5	None	2.5	P.E.	25
PALM RBD	5	None	2.5	P.E.	25

OIL	WATER (ml)	ALKANOL (ROH) R	AMOUNT ROH (ml)	WATER: ALKANOL RATIO	TIME (hr)	YIELD ESTERS
PALM RBD	100	n-butyl	50	2 : 1	1	20%
PALM RBD	100	n-butyl	50	2 : 1	2	30%
PALM RBD	100	n-butyl	50	2 : 1	3	50%
PALM RBD	100	n-butyl	50	2 : 1	4	60%
PALM RBD	100	n-butyl	50	2 : 1	5	60%
PALM RBD	100	n-butyl	50	2 : 1	6	70%
PALM RBD	100	n-butyl	50	2 : 1	7	80%
PALM RBD	100	n-butyl	50	2 : 1	25	100%

CLAIMS

1. A process for the preparation of an alkyl ester from a substrate selected from oils (as hereinbefore defined) and glyceride-containing fractions thereof, which comprises mixing a solution of the substrate in an inert organic solvent with an alkanol in the presence of a lipase and an excess (by weight) of water over the weight of substrate.

2. A process as claimed in Claim 1, wherein the substrate is a vegetable oil or a glyceride-containing fraction thereof.

3. A process as claimed in Claim 2, wherein the vegetable oil is coconut oil, corn oil, rapeseed oil, soyabean oil or sunflower oil.

4. A process as claimed in Claim 2, wherein the vegetable oil is palm oil.

5. A process as claimed in Claim 4, wherein the substrate is crude palm oil, crude palm kernel oil, crude palm stearin, palm fatty acid distillate, palm sludge oil, or refined, bleached and deodorized (RBD) palm oil.

6. A process as claimed in any one of the preceding claims, wherein the solvent is a water-

immiscible hydrocarbon, ether or ester.

7. A process as claimed in Claim 6, wherein the solvent is a methyl ester of palm oil or is petroleum ether.

8. A process as claimed in Claim 7, wherein the solvent is 60–80°C petroleum ether.

9. A process as claimed in any one of the preceding claims, wherein the solvent is present in an excess by weight of the substrate.

10. A process as claimed in Claim 9, wherein the solvent is present in an amount of at least 2 litres per kilogram of substrate.

11. A process as claimed in Claim 10, wherein the amount of solvent is 2 to 10 litres per kilogram of substrate.

12. A process as claimed in Claim 11, wherein the amount of solvent is 4 to 6 litres per kilogram of substrate.

13. A process as claimed in any one of the preceding claims, wherein the water and alkanol are present in a combined amount of at least 2 litres per kilogram of substrate.

14. A process as claimed in Claim 13, wherein the combined volume of water and alkanol is 15 to 40 litres per kilogram of substrate.

15. A process as claimed in Claim 14, wherein the combined volume of water and alkanol is 20 to 30 litres per kilogram of substrate.

16. A process as claimed in any one of the preceding claims, wherein the water content exceeds 40% by volume of the combined water and alkanol content.

17. A process as claimed in Claim 16, wherein the water content exceeds 50% by volume of said combined volume.

18. A process as claimed in Claim 17, wherein the water content is 50% to 90% by volume of said combined volume.

19. A process as claimed in any one of the preceding claims, wherein the alkanol has a carbon content of 1 to 8 carbon atoms arranged in a straight or branched chain configuration.

20. A process as claimed in Claim 19, wherein the alkanol is methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol or n-octanol.

21. A process as claimed in Claim 20, wherein the alkanol is methanol.

22. A process as claimed in any one of the preceding claims, wherein the process is carried out at temperature within the range 20 to 50°C.

23. A process as claimed in Claim 22, wherein the said temperature is 25 to 40°C.

24. A process as claimed in any one of the preceding claims, wherein the lipase is of the Candida group.

25. A process as claimed in Claim 24, wherein the lipase is Candida Cylindracea.

26. A process as claimed in any one of the preceding claims, wherein the lipase is immobilised by adsorption on a carrier.

27. A process as claimed in Claim 26, wherein the carrier is acid-treated Florisil (magnesium silicate).

28. A process as claimed in any one of the preceding claims, wherein the oil, alkanol and solvent are mixed together and the mixture added to a paste or suspension of the lipase in the water.

29. A process as claimed in Claim 1 and substantially as hereinbefore described in any of the Examples.

30. An alkyl ester whenever prepared by a process as claimed in any one of the preceding claims.

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